Protein logic: a statistical mechanical study of signal integration at the single-molecule level

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Abstract

Information processing and decision making is based upon logic operations, which in cellular networks has been well characterized at the level of transcription. In recent years however, both experimentalists and theorists have begun to appreciate that cellular decision making can also be performed at the level of a single protein, giving rise to the notion of protein logic. Here we systematically explore protein logic using a well known statistical mechanical model. As an example system, we focus on receptors which bind either one or two ligands, and their associated dimers. Notably, we find that a single heterodimer can realize any of the 16 possible logic gates, including the `XOR` gate, by variation of biochemical parameters. We then introduce the novel idea that a set of receptors with fixed parameters can encode functionally unique logic gates simply by forming different dimeric combinations. An exhaustive search reveals that the simplest set of receptors (two single-ligand receptors and one double-ligand receptor) can realize several different groups of three unique gates, a result for which the parametric analysis of single receptors and dimers provides a clear interpretation. Both results underscore the surprising functional freedom readily available to cells at the single-protein level.

Introduction

Cells depend on cues from their environment to initiate behaviors, including growth, division, differentiation, and death. Based upon these environmental signals cells must make decisions, such that the correct response is initiated. Although a particular environmental signal often elicits a particular cellular response, it is well established that signals can also act in combination (1–4). In this case the response triggered when two signals are present can be distinct from the responses triggered by each signal alone. The cell thereby acts as a logic gate, integrating two inputs to produce a single output. For example, the `AND` gate produces an output if both inputs are present, but it produces no output if either a single input or no input is present. For the process of decision making, the logic gate is the basic unit of computation, and therefore many studies have been devoted to its role within biochemical networks. Indeed, the role of logic gates within transcriptional networks has been studied in depth: systematic theoretical studies have predicted (5–8) and experimental studies have confirmed (9–12) that transcriptional networks can access all possible types of logic gate.

Recently, it has become clear that individual proteins can perform logic operations as well. Although this notion was initially suggested almost two decades ago (4), recent experiments have provided striking demonstrations. For example, performance of an `AND` gate by the actin regulatory protein N-WASP has been observed *in vivo* (3): when both of its inputs Cdc42 and PIP2 are present, they jointly "unfold" the active domain, leading to the activation of its target Arp2/3. Moreover, synthetic proteins based upon naturally existing proteins have been constructed and shown to perform a number of different logic operations (13, 14). Although these experiments beautifully illustrate the capacity for single proteins to encode logic, they are restricted to a limited set of logic gates. It therefore remains an open question if all possible logic gates can be accessed by single proteins, in particular the more complex gates like `XOR`, which includes nonmonotonic behavior.

Despite the fact that transcriptional logic has been explored in depth, to our knowledge no systematic theoretical study of protein logic has been done. A recent study focused on the nonmonotonic behavior of a single protein with several allosteric subunits, providing an understanding of how the action of a ligand as an agonist or an antagonist could be switched by the presence of a second ligand (15). Beyond this nonmonotonic behavior, however, other mappings of ligand presence to protein activity were not considered. By framing the problem as one of logic computation, we here obtain a comprehensive understanding of the functional mappings available to single proteins, thereby answering the question of which logical functions are possible, and under what conditions. Moreover, we use a less complex model than that used in (15), and we nonetheless find rich functional behavior, including nonmonotonicity, as characterized by the `XOR` gate.

For several reasons, we focus on receptor proteins, although our approach is easily extended to other protein types. First, receptors can be stimulated by multiple ligands (16–19), which naturally suggests a logic gate framework, in which multiple inputs (ligand concentrations) are integrated into one output (receptor activity). Second, receptors process signals directly at the plasma membrane. It is becoming increasingly recognized that the plasma membrane is a hub of information processing, acting as a mediator between the cell and its environment, along and across which signals are stored, processed, and relayed (20). Receptors are integral to this process, as they affect decisions directly at the detection level, before further intracellular transduction leads to the ultimate cellular response. The encoding of logic by receptors thus has the potential to be low-cost,

since it is achieved with a single protein, and rapid, since it occurs at the beginning of the signaling pathway. Finally, receptors often exist in the form of dimers or higher oligomers. For example, G protein-coupled receptors (GPCR) and ErbB receptors can each form dimers consisting of receptors of the same type (homodimers) or of receptors of different types (heterodimers) (16, 21, 22). A dimer has the capacity to perform the same or more logic operations than each of its monomeric constituents, a fact which we demonstrate here. Moreover, as we describe for the first time here, dimerization permits function space to be explored combinatorially: a cell can potentially change which logical function is performed simply by modulating which combination of monomers actually dimerizes.

We use a statistical mechanical model to develop a predictive framework for protein logic. We first numerically probe the logic gates accessible to individual receptor monomers and dimers by parameter variation, which has relevance on evolutionary timescales. We find that a single dimer can implement any of the possible logic gates with two inputs, a result which we support analytically. Next, we introduce the novel idea that a diverse set of logic gates can be performed, *not* by variation of parameters, but by modulating the dimerization of a fixed set of monomers. Such modulation may be achieved at the level of transcription and translation of monomeric proteins, or via post-translational modifications that enable monomers to dimerize; as such, we argue that dimeric recombination provides a way for a cell to modulate decisions on the timescales of gene expression or cell signaling. We find that the simplest set of receptors (two single-ligand receptors and one double-ligand receptor) can realize several different groups of three unique gates and that together these groups include all possible gates. We provide clear analytic support for this result, following the previous parametric analysis of single monomers and dimers. This result shines an interesting new light onto why receptors, or proteins in general, exist in the form of dimers. Both results underscore the surprisingly rich capacity for cells to encode decisions using single molecules. Finally, throughout the study, we discuss biological systems that implement these logical functions at the single-protein level.

Methods

We study receptor function by appealing to an equilibrium statistical mechanical model. Statistical mechanical models have been used quite fruitfully in the study of many molecular biology problems, including receptor activity and gene regulation (23). In the case of receptors, several models are well known. All assume that a receptor can exist in either an active (A) or an inactive (I) state, and that binding of a ligand changes the receptor bias for each state. In the Koshland-Nemethy-Filmer (KNF) model, ligand binding directly activates the receptor (24). That is, the bias is complete: a ligand-bound receptor is active, and an unbound receptor is inactive. This condition is relaxed in the Monod-Wyman-Changeux (MWC) model, in which ligand-bound receptors can be in either state, but coupled receptors switch between states in synchrony (25). Finally, in the conformational spread (CS) model (26, 27), both conditions are relaxed: a ligand-bound receptor can be in either state, and coupled receptors can be in different states. Because we are interested in the minimal model that can capture the ability to perform logic gates, we adopt the MWC model; the KNF model prohibits certain logic gates by construction, while the CS model allows excess parametric freedom (clearly, what can be achieved by the MWC model can be achieved by the CS model). Furthermore, the MWC model has been shown to agree with experiments on receptors (28–30).

The input in our model is the pair of concentrations $[S_1]$ and $[S_2]$ of two different ligands. The

output is the probability for a receptor monomer or dimer to be in its active state. We consider three monomer types and the associated dimers (Fig. 1): a monomer that binds ligand 1 (U), one that binds ligand 2 (V), and one that binds both ligands (W). In the last case, ligand binding is competitive: there is only one binding pocket, so only one ligand type can bind at a time. Noncompetitive binding, in which both ligand types can bind simultaneously, is captured by the Q_{UV} dimer (Fig. 1).

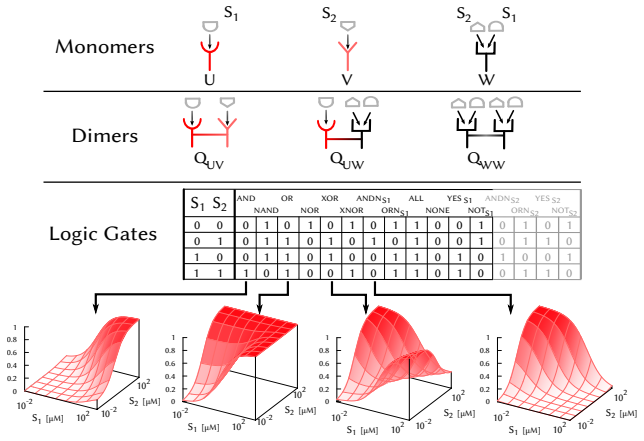

Figure 1: Setup. We consider receptor monomers (top row) that bind ligand 1 (U), ligand 2 (V), or both competitively (W), and their associated dimers (middle row). Bottom row: the table defines the 16 possible two-input logic gates in terms of binary input and output; below, for the four functionally unique gates, we plot the continuous analogs given by the statistical mechanical model.

The probability p^A for a receptor to be in the active state is computed from the partition functions, which enumerate all possible ways a receptor can be in either the active (Z^A) or inactive (Z^I) state:

$$p^A = \frac{Z^A}{Z^I + Z^A}. \tag{1}$$

The explicit forms of the partition functions under the MWC model are presented as each monomer and dimer is discussed in the Results section. For intuition, we provide an example here: the partition functions for monomer W are

$$Z^A = \omega_0 \left(1 + \frac{[S_1]}{K_1^A} + \frac{[S_2]}{K_2^A}\right), \tag{2}$$

$$Z^I = 1 + \frac{[S_1]}{K_1^I} + \frac{[S_2]}{K_2^I}, \tag{3}$$

where the parameter $\omega_0 = e^{-E_0/k_BT}$ is the Boltzmann factor corresponding to the energy difference E_0 between the active and inactive state, and the parameters K_i^j are the dissociation constants of ligand $i \in \{1, 2\}$ in activity state $j \in \{A, I\}$. The variables $[S_1]$ and $[S_2]$ are the ligand concentrations. In Eq. 2, the three terms correspond to the receptor being active when no ligand is bound,

when ligand 1 is bound, and when ligand 2 is bound, respectively. The same holds for Eq. 3 with the receptor being inactive.

The dependence of p_A on $[S_1]$ and $[S_2]$ defines the receptor's function (Fig. 1, bottom row). Functions are categorized based on the idealized behavior prescribed by the 16 possible two-input binary logic gates (Fig. 1). Mathematically, the function approaches binary logic when the output is either minimal ($p^A \to 0$) or maximal ($p^A \to 1$) in each of the four states defined by each input being absent ($[S_1] = 0$ or $[S_2] = 0$) or present ($[S_1] > 0$ or $[S_2] > 0$).

Numerically, when varying parameters to assess whether a receptor can realize a particular logic gate, we use a variant of the Wright-Fisher algorithm (31, 32), which models the evolution of a population. In the Wright-Fisher algorithm, evolution occurs in discrete, synchronous steps, and the population size remains constant. At each step, each member of the population produces offspring in proportion to its fitness. Then, mutations occur, and the mutated offspring comprise the population for the next step. In our case, for a given receptor, we have a "population" of R initial parameter points φ_r. Each point has fitness f_r, and the total fitness for the receptor is $F = \sum_r f_r$. At each step, R new points ("offspring") are drawn from the distribution $p_r = f_r/F$, which weights each point by its fitness. Each new point is then "mutated" by multiplying a randomly selected parameter by the factor $(1 + \delta)$, where δ is drawn uniform randomly from the range $[-\Delta : \Delta]$; we take $\Delta = 0.3$.

We define fitness as the agreement between the real-valued output of the statistical mechanical model p^A and the binary output of a specific ideal logic gate. The ideal logic gate is prescribed by the goal function $G([S_1], [S_2])$, which takes the value 0 or 1 depending on whether each input is "off" ($[S_1] < [S^*]$ or $[S_2] < [S^*]$) or "on" ($[S_1] > [S^*]$ or $[S_2] > [S^*]$), where we take the threshold value $[S^*] = 1\,\mu$M. We compare p^A and G over an $N \times N$ grid of input values, spaced logarithmically over the ranges of $[S_1]$ and $[S_2]$, which we take to be $[10^{-2} - 10^2]\,\mu$M. The fitness is thus

$$f_r = -\sum_{n,n'=1}^{N} \left| p^A\left([S_1^n], [S_2^{n'}]\right) - G\left([S_1^n], [S_2^{n'}]\right) \right|. \tag{4}$$

The results in this article are obtained for $N = 4$. Taking $N = 2$ leads to suboptimal results, while taking different values of $N > 2$ yields similar results to $N = 4$. Similar results are also obtained for a fitness function with $N = 2$ and an additional central point at $[S_1] = [S_2] = [S^*]$, at which G is the average of the truth table for the gate.

The optimization parameters, as well as the bounds within which the model parameters are initialized and constrained during optimization, are given in Table SI-1 in the Supporting Material. The chosen bounds fall within experimentally observed ranges and are consistent with typical values used in previous modeling studies; we elaborate upon this point in detail in Sec. SI.1.

When investigating whether multiple gates can be performed at fixed parameters by formation of the possible dimer combinations, we optimize for several logic gates at one time (Sec. SI.1). In practice, a given point in parameter space specifies both the dissociation constants K_i^j, which are intrinsic to each monomer U, V, and W and do not change when they are recombined, and the Boltzmann factors ω_0 and $\omega_{ii'}$, which are dimer-specific.

Results

First, we identify the logic gates that each receptor monomer and dimer can perform by parameter variation. Here, several derived analytic constraints support the numerical results. Then, we investigate the extent to which distinct logic gates can be formed using a set of monomers with fixed parameters by forming the possible dimer combinations. Several groups of distinct gates are possible, a finding for which the first results provide a clear interpretation.

Functions accessible by parameter variation

Figure 2 shows the set of logic gates that each monomer and dimer can perform, as determined by numerical optimization of model parameters. The most striking feature is that one of the dimers can perform all 16 possible gates. This and the other numerical results in Fig. 2 can be understood intuitively by appealing to analytic results derived from the underlying model, which we will describe in turn for each monomer and dimer.

Monomers

The first two monomers, receptors U and V, respond to only one input each. Therefore, they are trivially constrained to gates which depend on neither input (`ALL`, `NONE`) or on only one input ($\texttt{YES}_{S_i}$, $\texttt{NOT}_{S_i}$). Receptor W, on the other hand, allows competitive binding of both inputs, and can therefore realize several nontrivial gates.

At this point it is useful to observe that the gates exist in antagonistic pairs (a gate and its inverse), shown consecutively in Fig. 2: (`AND`, `NAND`), (`OR`, `NOR`), etc. Any receptor that can perform one member of a pair can perform the other, simply by inverting certain parameter values. Furthermore, several gates are equivalent under reversal of the two ligands (those with subscripts in Fig. 2): ($\texttt{ANDN}_{S_1}$, $\texttt{ANDN}_{S_2}$), ($\texttt{ORN}_{S_1}$, $\texttt{ORN}_{S_2}$), etc. Again, any receptor that can perform one of these can perform the other, simply by switching certain parameter values (corresponding to exchanging the effect of S_1 and S_2). Eliminating these redundancies, we arrive at four unique gates that respond nontrivially to both inputs:

$$\texttt{AND}, \quad \texttt{OR}, \quad \texttt{ANDN}_{S_1}, \quad \texttt{XOR}. \tag{5}$$

We will consider only these four unique gates from this point on.

The third monomer, receptor W, whose partition functions are given in Eqs. 2-3, can realize two of the four unique gates: `OR` and $\texttt{ANDN}_{S_1}$. The `OR` gate follows straightforwardly from the situation where both ligands activate the receptor individually; their combination will then activate it as well. The $\texttt{ANDN}_{S_1}$ gate can be formed if ligand 1 binds more strongly than ligand 2 ($K_1^j \ll K_2^j$), but ligand 1 only weakly biases the receptor toward the active state ($K_1^A \sim K_1^I$), while ligand 2 strongly biases it ($K_2^A \ll K_2^I$). In this scenario, a receptor that is inactive in the absence of both ligands ($\omega_0 \ll 1$) will only be active in the presence of ligand 2 *and not* 1.

We note here that any receptor that is activated by two different ligands is a biological example of an `OR` gate, and many naturally occurring receptors are activated by different ligands, like the TAR receptor (19) and the EGF receptor (33). More generally, it has been shown that proteins can be synthesized with a number of specific ligand-binding sites (34); such constructs can be thought of as extensions of the `OR` gate to more than two inputs. Additionally, the `ORNOT` gate, the inverse

of the ANDN gate, has been constructed synthetically using a single protein (construct H2, Fig. 2B in (14)).

Receptor W cannot realize the other two unique gates, AND and XOR. Both gates require a cooperative effect when both ligands are present: in the AND gate, neither ligand activates the receptor individually, but both activate it together; in the XOR gate, each ligand activates the receptor individually, but both suppress activation together. Such cooperative effects are not possible with competitive binding. As we will see next, dimerization is required to perform these gates.

Dimers

The three monomers admit six possible dimer combinations — three homodimers and three heterodimers. The homodimers Q_{UU} and Q_{VV} respond to only one input each and are therefore trivially constrained like monomers U and V. Moreover, heterodimers Q_{UW} and Q_{WV} are equivalent upon ligand exchange and can therefore realize equivalent sets of logic gates upon parameter variation. This leaves three dimers that can realize unique sets of logic gates upon parameter variation: Q_{UV}, Q_{UW}, and Q_{WW}.

The first dimer, receptor Q_{UV}, is the simplest heterodimer: it is formed by combining monomer U, which responds only to ligand 1, and monomer V, which responds only to ligand 2. Unlike receptor W, which is limited to competitive binding, the dimeric receptor Q_{UV} has two binding pockets and therefore allows noncompetitive (i.e. cooperative) binding. Accordingly, its partition functions extend those of receptor W (Eqs. 2-3) to include a cooperative term:

$$Z^A = \omega_0 \left(1 + \frac{[S_1]}{K_1^A} + \frac{[S_2]}{K_2^A} + \overbrace{\omega_{12} \frac{[S_1]}{K_1^A} \frac{[S_2]}{K_2^A}}^{\text{cooperative}} \right), \tag{6}$$

$$Z^I = 1 + \frac{[S_1]}{K_1^I} + \frac{[S_2]}{K_2^I} + \omega_{12} \frac{[S_1]}{K_1^I} \frac{[S_2]}{K_2^I}, \tag{7}$$

The cooperative term contains an additional Boltzmann factor $\omega_{12} = e^{-E_{12}/k_B T}$ corresponding to the cooperative binding energy E_{12}, which could originate from, e.g., a conformational change of the receptor upon binding of one ligand that opens the binding pocket for the other ligand. For example, the binding affinity of each of the inputs to the protein N-WASP is increased by a factor of ∼300 when the other input is bound (3).

Receptor Q_{UV} can realize three of the four unique gates: OR, $\mathtt{ANDN}_{S_1}$, and AND. The OR and $\mathtt{ANDN}_{S_1}$ gates follow straightforwardly from the fact that Q_{UV} reduces to W for no cooperativity ($\omega_{12} = 0$), and receptor W can realize these gates as previously discussed. The AND gate is formed when the receptor is inactive in the presence of each ligand alone but, due to the cooperative interaction, is active in the presence of both ligands together. Receptor Q_{UV} cannot realize the XOR gate: if the receptor is activated by either one of the two ligands, it must also be activated by both ligands together. The cooperative interaction enhances the effect that each ligand individually has on the activation of the receptor, but it cannot reverse it.

The intuition behind why receptor Q_{UV} can realize the AND gate can be quantified by considering the constraints that an AND gate places on the partition functions:

$[S_1]$	$[S_2]$	p^A	
0	0	0	$\omega_0 \ll 1$
$[\bar{S}_1]$	0	0	$\omega_0 \left(1+\frac{[\bar{S}_1]}{K_1^A}\right) \ll 1+\frac{[\bar{S}_1]}{K_1^I}$
0	$[\bar{S}_2]$	0	$\omega_0 \left(1+\frac{[\bar{S}_2]}{K_2^A}\right) \ll 1+\frac{[\bar{S}_2]}{K_2^I}$
$[\bar{S}_1]$	$[\bar{S}_2]$	1	$\omega_0 \left(1+\frac{[\bar{S}_1]}{K_1^A}+\frac{[\bar{S}_2]}{K_2^A}+\omega_{12}\frac{[\bar{S}_1][\bar{S}_2]}{K_1^A K_2^A}\right) \gg 1+\frac{[\bar{S}_1]}{K_1^I}+\frac{[\bar{S}_2]}{K_2^I}+\omega_{12}\frac{[\bar{S}_1][\bar{S}_2]}{K_1^I K_2^I}$

(8)

Here, $[\bar{S}_1]$ and $[\bar{S}_2]$ denote the maximum input values. We have recognized that a low output requires $Z^A \ll Z^I$ (see Eq. 1); therefore, the first three lines reflect that in an `AND` gate the output is low in the first three input conditions. Similarly, a high output requires $Z^A \gg Z^I$, which is reflected in the last line. Receptor Q_{UV} can realize the `AND` gate precisely because the constraints in Eq. 8 can be met simultaneously. For example, taking for illustration the simplifying case of intermediate cooperativity ($\omega_{12} \gtrsim 1$) and symmetric, saturating ligand concentrations ($[\bar{S}_1]/K_1^j = [\bar{S}_2]/K_2^j \gg 1$), Eq. 8 reduces to

$$1 \ll 1/\sqrt{\omega_0} \ll K_1^I/K_1^A \ll 1/\omega_0. \tag{9}$$

Indeed, we see that the `AND` gate requires a bias upon ligand binding that is too weak to activate the receptor individually ($K_1^I/K_1^A \ll 1/\omega_0$), but strong enough to activate the receptor cooperatively ($K_1^I/K_1^A \gg 1/\sqrt{\omega_0}$).

The strength of the cooperativity influences the quantitative properties of the `AND` gate: an increase in ω_{12} shifts the transition region of the gate to smaller ligand concentrations, as indeed observed in studies of the `AND`-like N-WASP protein (3).

In addition to N-WASP (3), the `AND` gate logic is observed in various other proteins. For example, in gonadotropes, the scaffold PEA-15 is activated only by the simultaneous presence of PKC and ERK (35). Similarly, the adaptor protein TIRAP functions as a coincidence detector (36), thereby only becoming activated when two inputs are present at the same time. In *V. Harveyi*, coincidence detection is also exhibited for the two quorum signals AI-1 and AI-2 (37).

The second dimer, receptor Q_{UW}, is also a heterodimer: it is formed by combining monomer U, which responds only to ligand 1, and monomer W, which responds competitively to both ligands. The partition functions for this receptor are

$$\begin{aligned} Z^A = \omega_0\Big(1 &+ \frac{[S_1]}{K_{1,U}^A} + \frac{[S_1]}{K_{1,W}^A} + \frac{[S_2]}{K_2^A} \\ &+ \omega_{11}\frac{[S_1]^2}{K_{1,U}^A K_{1,W}^A} + \omega_{12}\frac{[S_1]\,[S_2]}{K_{1,U}^A K_2^A}\Big), \end{aligned} \tag{10}$$

$$\begin{aligned} Z^I = 1 &+ \frac{[S_1]}{K_{1,U}^I} + \frac{[S_1]}{K_{1,W}^I} + \frac{[S_2]}{K_2^I} \\ &+ \omega_{11}\frac{[S_1]^2}{K_{1,U}^I K_{1,W}^I} + \omega_{12}\frac{[S_1]\,[S_2]}{K_{1,U}^I K_2^I}. \end{aligned} \tag{11}$$

Here, since ligand 1 can bind to either monomer U or W, we distinguish these cases with the second subscript on K_1^j. There are now two cooperative terms, corresponding to the cases where monomers U and W bind, respectively, ligands 1 and 1 (ω_{11}), or ligands 1 and 2 (ω_{12}). Eqs. 10-11 make clear that receptor Q_{UW} reduces to receptor W (Eqs. 2-3) in the limit $K_{1,U}^j \to \infty$, and to receptor Q_{UV} (Eqs. 6-7) in the limit $K_{1,W}^j \to \infty$.

Receptor Q_{UW} can realize all four unique gates (and therefore all 16 possible gates; Fig. 2). The `OR`, $\texttt{ANDN}_{S_1}$, and `AND` gates follow straightforwardly from the fact that receptor Q_{UV}, which can realize these gates, is a limiting case. The `XOR` gate is less trivial. Below we offer an intuitive argument for why receptor Q_{UW} can realize an `XOR` gate, and in Sec. SI.2 we prove analytically that the output can be a nonmonotonic function of the two inputs for this receptor, which is required for an `XOR` gate.

The `XOR` gate is formed when each ligand individually activates the receptor by binding to monomer W, but when both ligands are present, ligand 1 is outcompeted and thus binds to monomer U, in turn suppressing activation. It is instructive here to describe this process in more detail. Suppose that ligand 1 promotes activation when bound to W but suppresses activation when bound to U. Further, suppose that ligand 1 binds more strongly to W than to U, such that in the presence of ligand 1 alone, the receptor is active. Now suppose that ligand 2 promotes activation when bound to W. Since ligand 2 can only bind to W, in the presence of ligand 2 alone, the receptor is also active. Finally, suppose that ligand 2 "interferes" with ligand 1, i.e. binds more strongly to W than ligand 1 does. Then, in the presence of both ligands, ligand 2 binds to W, leaving ligand 1 to bind to U. If U suppresses activation more strongly than W promotes activation, then in the presence of both ligands, the receptor is inactive. The resulting logic is the `XOR` gate.

The third dimer, receptor Q_{WW}, is a homodimer: it is formed by combining two W monomers, each of which responds competitively to both ligands. The partition functions for this receptor are

$$\begin{aligned} Z^A = \omega_0\Big(1 + 2\frac{[S_1]}{K_1^A} + 2\frac{[S_2]}{K_2^A} \\ + \omega_{11}\frac{[S_1]^2}{K_1^A K_1^A} + 2\omega_{12}\frac{[S_1]\,[S_2]}{K_1^A K_2^A} + \omega_{22}\frac{[S_2]^2}{K_2^A K_2^A}\Big), \end{aligned} \tag{12}$$

$$\begin{aligned} Z^I = 1 + 2\frac{[S_1]}{K_1^I} + 2\frac{[S_2]}{K_2^I} \\ + \omega_{11}\frac{[S_1]^2}{K_1^I K_1^I} + 2\omega_{12}\frac{[S_1]\,[S_2]}{K_1^I K_2^I} + \omega_{22}\frac{[S_2]^2}{K_2^I K_2^I}. \end{aligned} \tag{13}$$

Here, the factors of two account for the fact that each ligand can be bound to either of two symmetric monomers. There are now three cooperative terms, corresponding to the cases where both monomers bind ligand 1 (ω_{11}), both bind ligand 2 (ω_{22}), or one binds ligand 1 and the other binds ligand 2 (ω_{12}).

Receptor Q_{WW} can realize three of the four unique gates: `OR`, $\texttt{ANDN}_{S_1}$, and `AND`. The `OR` and $\texttt{ANDN}_{S_1}$ gates follow straightforwardly from the fact that each monomer alone can realize these gates as previously discussed. The `AND` gate relies on strong suppression of cooperation between monomers if they are bound to the same ligand type (i.e. $\omega_{11} \to 0, \omega_{22} \to 0$); this suppression prevents activation when only one ligand is present. In fact, this limit reduces Eqs. 12-13 to Eqs. 6-7 (up to factors of 2), meaning the `AND` gate constraint, Eq. 9, also holds here under the same

conditions for which it was derived. Receptor Q_{WW} cannot realize the `XOR` gate: because the individual monomers are identical, no negative interference is possible, as it is for receptor Q_{UW}.

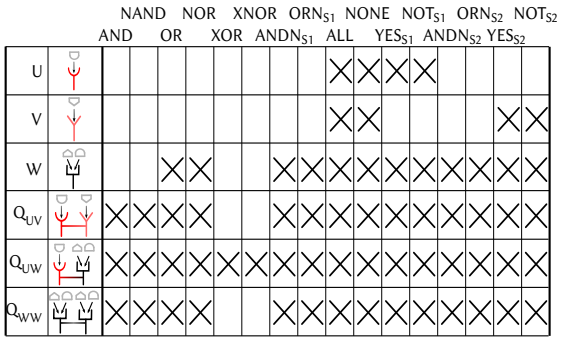

Figure 2: Functional versatility by parameter variation. For all monomers and dimers, we show the possible functions attainable by varying parameters. Attainability is assessed by numerical optimization and interpreted based on analytic constraints derived in the text.

Functions accessible by recombination

In the previous section we identified the logic gates accessible by individual receptors via variation of intrinsic biochemical parameters. In this section we ask a separate question. We here seek the logic gates that a set of monomers can realize — at fixed parameters — simply by forming the possible dimer combinations. This question is critically related to the challenge that all cells face: to encode reliable responses using limited resources (here, a limited set of monomers) and on short timescales (here, set by gene expression and cell signaling). This question is also key to functional control at the single-protein level: if diverse logic gates can be realized by a small set of monomers, cellular function could be strongly tuned in a straightforward manner, e.g. by expressing a particular pair of monomers and not others.

The three monomers we study form four functional dimers: Q_{UV}, Q_{UW}, Q_{WV}, and Q_{WW} (the dimers Q_{UU} and Q_{VV} respond to only one input each and are neglected). This fact leads to the enticing question of whether there exist parameters at which the four dimers perform the four unique logic gates (Eq. 5). Such a finding would be highly nontrivial: all monomers are present in at least two dimers, and therefore the performance of a particular logic gate by one dimer places heavy constraints on the parameters of the other dimers.

An exhaustive search, in which we numerically optimize for each of the $4! = 24$ dimer-to-logic gate mappings in turn (Sec. SI.1), suggests that no parameter set exists at which all four unique logic gates are performed. Moreover, replacing any subset of gates with the corresponding inverse gates and repeating gives the same result in each of the $2^4 = 16$ cases. Interestingly, the result seems to be due to the fact that the parameters which support the `XOR` gate in receptor Q_{UW} (or its counterpart Q_{WV}) prohibit the `AND` gate in any of the other receptors. Next we support this numerical observation with an intuitive argument.

Suppose that receptor Q_{UW} performs an `XOR` gate. As described in the previous section, the `XOR` gate requires that when ligand 1 is present alone, it activates the receptor by binding to monomer

W. Since the `AND` gate requires the opposite behavior, namely that the receptor is inactive when ligand 1 is present alone, then the `AND` gate cannot be formed by any receptor in which ligand 1 only binds to W. This group includes receptors Q_{WW} and Q_{WV}, leaving only receptor Q_{UV}. Then, as also described in the previous section, the `XOR` gate requires that ligand 1 suppresses activation when bound to monomer U. Since the `AND` gate requires that the receptor is active when both ligands are present, in receptor Q_{UV} this suppression would have to be overpowered by activation via ligand 2 binding to V. However, if this were the case, the receptor would surely be active in the presence of ligand 2 alone, which is inconsistent with the behavior of an `AND` gate. These arguments make clear that if receptor Q_{UW} performs an `XOR` gate, no other receptor can form an `AND` gate. The same arguments, but with the ligands exchanged, hold if receptor Q_{WV} performs the `XOR` gate instead of receptor Q_{UW}. Since receptors Q_{UW} and Q_{WV} are the only receptors that can perform the `XOR` gate, we conclude that the `XOR` and `AND` gates are not mutually accessible by recombination of monomers U, V, and W at fixed parameters.

Even though all four unique logic gates cannot be performed at fixed parameters, we do find six parameter sets at which unique groups of three logic gates are performed by three of the dimers. We denote these parameter sets as φ_k, for $k \in \{1, 2, \ldots, 6\}$, and show the logic gates and the dimers that perform them in Fig. 3. We stress that this result is still nontrivial: two of the groups are performed by receptors Q_{WW}, Q_{UW}, and Q_{WV}, which all contain monomer W; additionally, two groups are performed by receptors Q_{UW}, Q_{WV}, and Q_{UV}, in which each monomer is represented in two of the three dimers. Due to the high degree of monomer overlap in both cases, one might have expected the three dimers to be constrained to similar functionality at fixed parameters; instead, we find that three unique logic gates can be formed. Further, Fig. 3 reveals that all four logic gates are represented among the six groups (but, as expected, never `XOR` and `AND` in the same group). Finally, the optimal solutions shown in Fig. 3 are robust to parametric perturbation: as shown in Sec. SI.3, for all φ_k, most random perturbations in which each parameter changes by an average of $\sim$20% change the fitness of none of the three logic gates by more than 10%. All of these features underscore the functional versatility available to cells by dimeric recombination.

Our finding that cells can perform multiple logic gates at fixed parameters naturally raises the question of whether the gates conflict with each other, which could potentially corrupt the computation. Moreover, since we imagine that the dimers are present on the membrane in quasi-equilibrium with their monomeric constituents, we must also consider whether the gates are in conflict with the logic encoded by the monomers themselves. This latter question is straightforward to resolve. First, the monomers U and V respond to only one input each and therefore do not perform nontrivial logic gates. Second, while the monomer W can perform one of two nontrivial logic gates (`OR` or `ANDN`), the dimer Q_{WW} then also performs this gate. Any conflict between W and a dimer therefore also arises as a conflict between Q_{WW} and that dimer. We thus consider only conflict between dimers from here on.

One simple way of minimizing conflict between dimers is by selectively expressing only a particular pair of monomers and not the other monomer (Fig. 4a). For example, at parameter set φ_3 (see Fig. 3), if monomers U and V were expressed, but not W, the only dimer that could form is Q_{UV}, resulting in the unambiguous encoding of an `ANDN` gate. If at some later time, monomers U and W were expressed, but not V, only Q_{UW} and Q_{WW} could form; then, since Q_{WW} is not functional at φ_3, the `XOR` gate would be encoded unambiguously. Similarly, expression of V and W but not U would encode the `OR` gate unambiguously. The time between these periods of selective expression would be set by gene expression and would therefore be long compared to the timescale on which

the cell actually employs the logic gate to respond to the incoming signals. We observe from Fig. 3 that both parameter sets φ_3 and φ_4 share the property that all three gates can be encoded unambiguously by selective expression; in this sense they are "optimal" in terms of minimizing conflict between gates. By contrast, the other parameter sets suffer from conflict between Q_{UW} and Q_{WW} when only U and W are expressed (φ_1, φ_2, φ_5, φ_6), or between Q_{VW} and Q_{WW} when only V and W are expressed (φ_1, φ_2).

Ultimately, the most general solution to the problem of dimer conflict — and indeed, one that is commonly exploited by cells — is to make the downstream response dimer-specific. Specificity can be established in several ways. The immediate downstream component can respond preferentially to one dimer and not to another, as observed for the EGF receptor family (38). The specificity could then be propagated further downstream, for example at the level of transcriptional regulation (Fig. 4b). Alternatively, specificity can be achieved via spatial segregation of membrane components (Fig. 4c). For example, interaction with lipid rafts is thought to separate membrane proteins into spatially distinct, non-mixed clusters, leading to added specificity in downstream computations (39, 40). Either of these mechanisms would allow several types of dimers to coexist on the membrane and control, simultaneously and without conflict, distinct downstream processes according to distinct logical functions.

In the remainder of this section, we provide for parameter sets φ_1 and φ_2 the intuition behind how the three logic gates in Fig. 3 are performed by the corresponding receptors. In Sec. SI.4, we provide similar intuition for parameter sets φ_3, φ_4, φ_5, and φ_6. Furthermore, in Sec. SI.4, we argue why the groups observed in Fig. 3 (and their counterparts obtained upon ligand exchange) are the *only* groups of three unique logic gates that one expects to observe under this model.

Parameter set φ_1 (Fig. 3, first row) corresponds to a case where each ligand only weakly promotes activation in the presence of monomer W. This feature allows receptor Q_{WW} to remain inactive when each ligand is present individually but become activated when both ligands are present together, forming the `AND` gate. Furthermore, ligand-bound U both promotes activation and strongly enhances the binding of ligand 2 to W. This feature allows receptor Q_{UW} to perform the `OR` gate: when ligand 1 is present alone, it promotes activation by binding to U; when ligand 2 is present in abundance and ligand 1 is present only in a small amount (and thus still in the "off" state), the small amount of ligand 1 is nonetheless sufficient to promote activation via enhanced binding of ligand 2 to W; and when both ligands are present in abundance, the two effects combine, resulting in activation. Finally, ligand-bound V both suppresses activation and strongly enhances the binding of ligand 1 to W. This feature allows receptor Q_{WV} to perform the $\texttt{ANDN}_{S_2}$ gate: when ligand 2 is present it suppresses activation via V, independent of ligand 1; but when ligand 1 and not (very much of) ligand 2 is present, the small amount of ligand 2 strongly enhances binding of ligand 1 to W, thus promoting activation.

Parameter set φ_2 (Fig. 3, second row) corresponds to a case where ligand-bound W promotes activation. This feature is sufficient for receptor Q_{WW} to perform the `OR` gate. Furthermore, ligand 2 binds more strongly to V than to W, and ligand-bound V suppresses activation more strongly than ligand-bound W promotes activation. These features allow receptor Q_{WV} to perform the $\texttt{ANDN}_{S_2}$ gate, since only in the presence of ligand 1 and not 2 will activation be promoted via W and not suppressed via V. Finally, (i) ligand 1 binds more strongly to W than to U, (ii) ligand 2 binds more strongly to W than ligand 1 does, and (iii) ligand-bound U suppresses activation more strongly than ligand-bound W promotes activation. These are the precise features that allow receptor Q_{UW} to perform the `XOR` gate, as outlined in detail in the previous section.

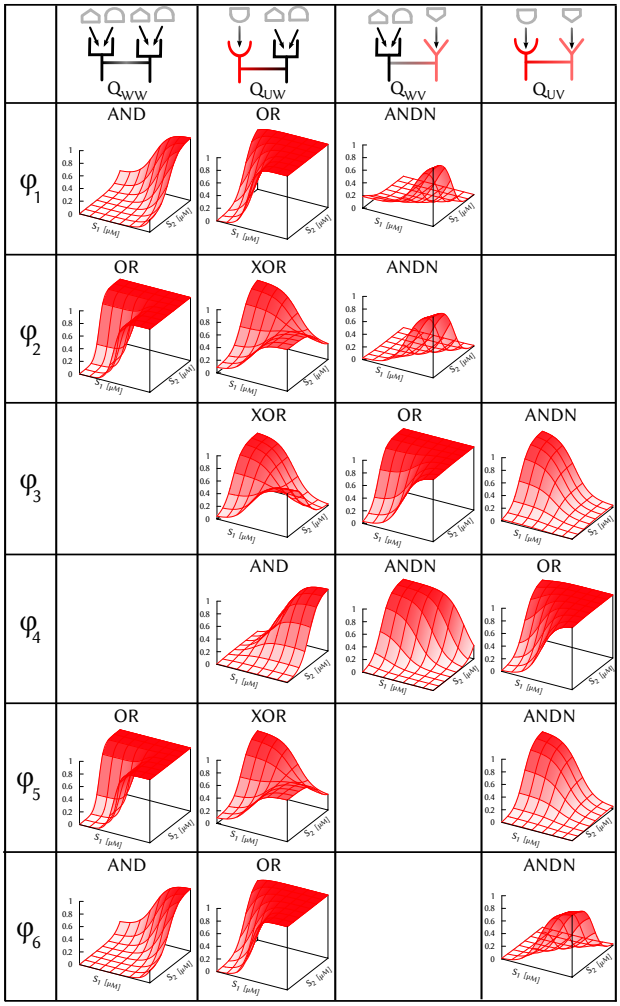

Figure 3: Functional versatility by recombination. Given the three monomer types, four functional units can be formed by dimerization. Six parameter sets φ_k are shown at which three of the four dimers perform functionally unique logic gates.

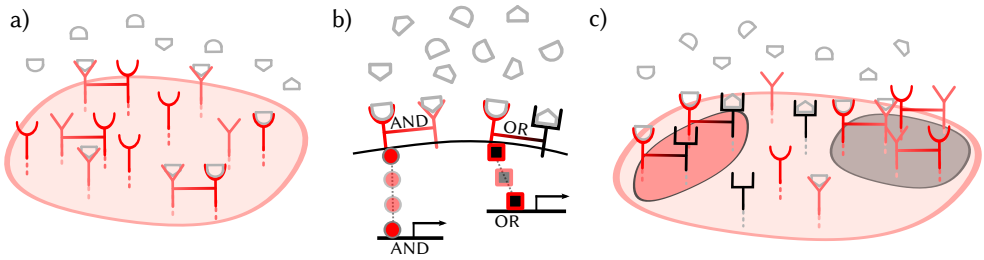

Figure 4: Several established mechanisms can minimize conflict between dimers' logical functions. (a) Selective expression of only two of the three monomers allows the formation of only one functional dimer, while (b) specificity of the downstream component or (c) spatial segregation of membrane components allows multiple functional dimers to coexist without conflict.

Discussion

We have used a statistical mechanical model to investigate the versatility of receptor function in two contexts: (i) the ability of a single receptor to access logical functions by parameter variation, and (ii) the ability — at fixed parameters — for a set of receptor monomers to access logical functions by dimerizing. The first context is important on evolutionary timescales, on which mutations and environmental pressures act to change a cell's intrinsic biochemical parameters. The second context is more critical at far shorter timescales, i.e. timescales characterizing the response of individual cells, during which gene expression and covalent modification can potentially change cellular function at the molecular level by favoring the dimerization of particular receptors over others.

In the first context, we find that a single heterodimer (receptor Q_{UW}) can realize all possible logic gates by parameter variation. Our analysis reveals that such complete functional freedom, while perhaps surprising, is in fact quite intuitive for this receptor. In particular, receptor Q_{UW} performs the most challenging function, the `XOR` gate, by exploiting an interference between the two ligands (i.e. when both ligands are present, one outcompetes the other for the activating binding pocket, ultimately causing suppression). Such a nonmonotonic response requires competitive binding and asymmetric activation biases, both of which are possible by heterodimerization.

In the second context, we find that the simplest combination of monomers that yields four functional dimers cannot in fact perform the four unique logic gates at fixed parameters, an observation we explain by arguing that the `AND` gate and the `XOR` gate are not mutually accessible. Nonetheless, numerical search reveals that several distinct groups of three unique gates are performable, a result that is nontrivial given the high degree of overlap among dimers' parameter spaces. We offer intuitive explanations for the emergence of these groups, and further, we argue that these groups are exhaustive. The ability to perform diverse functions with a limited set of simple components is of critical importance to the question of how cells encode reliable responses with limited resources.

Although we often think of logic functions as the fundamental units of decision making, logic operations reduce the output space to a binary variable. In principle the full input-output relation, which conveys much more information, could be used to regulate downstream responses. Indeed, the output of our statistical mechanical model is not restricted to Boolean logic, but rather constitutes continuous response functions. However, we argue that the most simple form of transducing

information on ligands is via an input-output relation that approximates a binary response. In fact, recent experiments have shown that the information transmission capacity of a receptor is indeed approximately 1 bit, which is equivalent to a binary response (41).

We have adopted a minimal model (the MWC model) to describe a minimal set of components, and we have explored the functional capabilities available under these conditions. We are further encouraged by the fact that the MWC model has been shown to agree with experiments on receptors (28–30). Nonetheless, several extensions to the model or the study itself are natural choices for further exploration. First, the conformational spread (CS) model (26, 27) generalizes the MWC model, and thus it would allow for more functional freedom in logic gate construction. However, it is always a concern that generalizing one's model can reduce the fraction of functional parameter space simply by increasing the total volume of parameter space. Second, it would be straightforward to introduce one or more additional monomers when considering recombination. For example, introducing an additional monomer that binds a single ligand might in fact admit parameter sets at which all four unique logic gates are performed, at the expense of increasing the number of individual components that the cell must produce. The impact of such a finding, however, would be reduced by the fact that more than four functional dimer combinations would be possible. Third, it would also be straightforward to consider more complex dimers (or higher oligomers), such as $Q_{W_1W_2}$, in which each pocket binds both ligands competitively, but with asymmetric parameters. Of course, such increasing complexity would only be justified in the context of correspondingly detailed biological examples.

It is well established that receptors are responsive to multiple ligands. Recent experiments have indeed exploited this fact to synthetically construct proteins that perform a limited set of logic gates (42). At the same time, observations of oligomerization and protein modification on the membrane suggest that receptors can act as functional signaling units by recombination. Indeed, experiments have shown that for many receptors, such as ErbB and GPCR, monomers combine to form different dimers that have different functionality (16, 21, 22). We anticipate that this study will contribute to a predictive framework in which experiments like these can be interpreted and extended. The findings we report — that a single receptor can function as any logic gate and that a limited set of monomers can access diverse logic gates by dimerizing — speak to the large degree of functional control available to cells at the level of individual receptor molecules.

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SI. 1 Optimization details

Optimization parameters

In Table SI-1 we provide bounds for our optimization parameters K_i^j, ω_0 and $\omega_{ii'}$. In this section we provide experimental support for the chosen bounds.

- ω_0
  Two experimental studies report on explicit values of ω_0. In the quorum sensing bacterium *V. Harveyi* an ω_0 value is reported of $e^{-\Delta\epsilon/k_BT} = e^{3.2} \approx 24$ (43). Next, a conformational spread model for the motor proteins in *E. coli* reported for $\omega_0 = e^{-E_A/k_BT} = e^{-0.66} = 0.51$ (44). Moreover, two modeling studies have also suggested values for ω_0. In (45) a value of $\omega_0 = e^{-E_A/k_BT} = 0.36$, while in (15) a large range for ω_0 is suggested $\omega_0 \sim [e^{-10} - e^{10}]$.

- $\omega_{ii'}$
  For *E. coli* different experimental observations are reported, both for the motor proteins and the receptors. The receptor coupling energy has been reported to be around 0 k_BT, leading to $\omega_{ii'} = 1$ (28, 29). For the motor protein, a value for $\omega_{ii'} = e^{E_{ii'}/k_BT} = 62$ has been reported (44). In a modeling study of the protein N-WASP $\omega_{ii'} \sim 300$ has been used (3). In the EGFR receptor for monomers $K_D \sim 10^0$ [nM], while for dimers $K_D \sim 10^{-2}$ [nM], suggesting a positive coupling between two monomers ($\omega_{ii'} > 1$) (33). In the same modeling studies as cited above, estimates are $E_j = \ln \omega_{ii'} =$ 0-4 k_BT (45) and a large range $\omega_{ii'} \sim [e^{-10} - e^{15}]$ (15).

- K_i^j
  In *E. coli* dissociation constants for different ligands and activation states for the Tar and Tsr receptors have been measured which vary between $[10^{-2} - 10^6]$ [mM] (28, 29). Experimental work on the receptors of the quorum sensing machinery in *V. Harveyi* where single amino-acids are replaced have resulted in dissociation constant varying between $[10^0 - 10^5]$ [nM] (43). For EGFR different K_D's are reported for different dimer pairs, ranging from 10 [pM] to 500 [nM] (21, 33). Synthetic proteins are constructed with varying dissociation constants for different ligands, where the K_D ranges from $[1 - 1000]$ [μM] (14) or $[10^{-1} - 10^5]$ [μM] (34). A mathematical model based upon a three-state receptor with multiple ligands used K_D values from $[10^{-9} - 10^{-5}]$ [μM] for each ligand and state of the system (46).

Multiple receptors

In the case where M different receptors (e.g. $Q_{\mathrm{UV}}, Q_{\mathrm{WV}}, Q_{\mathrm{UW}}$) are combined to act in different combinations as unique logic gates, the optimization algorithm follows a specific order in the optimization. A straightforward extension of the model for a single receptor is the optimization of three (or four) gates simultaneously, and taking as fitness $\mathcal{F}$ the summed fitness of every gate F_m:

$$\mathcal{F} = \sum_{m=1}^{M} F_m. \tag{SI-1}$$

However, this optimization is not capable of optimizing all the gates independently. Instead, the algorithm optimizes either one (or two) gates, but then cannot optimize the third gate. To optimize

Model input	**Range**
$[S_1], [S_2]$	$\left[10^{-2} - 10^{2}\right]$ μM
Model parameter	**Bounds**
$K_{i,k}^{j}$	$\left[10^{-3} - 10^{4}\right]$ μM
ω_0	$\left[10^{-3} - 10^{3}\right]$
$\omega_{ii'}$	$\left[10^{-2} - 10^{2}\right]$
Optimization parameter	**Value**
Δ	0.3
N	4
R	50000
Steps	1000

Table SI-1: Overview of parameters. During optimization, model parameters are initialized and constrained within the indicated bounds.

the third gate, the already optimized gates decrease (temporarily) in fitness. This decrease is larger than the increase in fitness for the third gate and the algorithm finds suboptimal peaks in this rugged fitness landscape.

Instead of optimizing all gates simultaneously, we optimize gates in order. For the homodimer construction ($Q_{\mathrm{WW}}, Q_{\mathrm{UW}}, Q_{\mathrm{WV}}$), we first optimize Q_{WW}, then Q_{UW}, where we only change the parameters of U, and then Q_{WV}, only changing V. The achieved results greatly outperform the results where we optimize all three gates simultaneously.

For the heterodimer construction ($Q_{\mathrm{UW}}, Q_{\mathrm{WV}}, Q_{\mathrm{UV}}$), we again start by optimizing gate Q_{UW}, then the two gates Q_{UW} and Q_{WV} simultaneously, and finally Q_{UW}, Q_{WV}, and Q_{UV}. Again this procedure gives much better results than simultaneous optimization of all three gates.

For the construction with $Q_{\mathrm{WW}}, Q_{\mathrm{UW}}, Q_{\mathrm{UV}}$), we start by optimizing gate Q_{WW}, then the two gates Q_{WW} and Q_{UW} simultaneously, and finally Q_{WW}, Q_{UW}, and Q_{UV}.

SI. 2 Formal proof that receptor Q_{UW} can perform an `XOR` gate

The probability to be active $p^A\left([S_1], [S_2]\right)$ in an `XOR` gate is a nonmonotonic function of $[S_1]$ and $[S_2]$ simultaneously. More specifically, for constant $[S_2] = [S_2^c]$, $p^A\left([S_1], [S_2^c]\right)$ is either monotonically increasing or decreasing, depending on the value $[S_2^c]$: for small $[S_2^c]$, p^A is monotonically increasing, while for large $[S_2^c]$, p^A is monotonically decreasing.

A positive derivative $\partial p^A/\partial[S_2]$ reflects a monotonically increasing function, while a negative derivative reflects a monotonically decreasing function. Therefore, in an `XOR` gate, the derivative of the probability with respect to $[S_2]$ at constant $[S_1^c]$ should change sign as function of $[S_1^c]$. Again due to symmetry, the derivative of the probability with respect to $[S_1]$ at constant $[S_2^c]$ should change sign as function of $[S_2^c]$. We will prove that the `XOR` gate is possible for the Q_{UW} receptor even with $\omega_{11} = \omega_{21} = 1$. Recalling Eq. 1, the derivative can be written $\partial p^A/\partial[S_2] = f/(Z^A + Z^I)^2$, where the numerator

$$f\left([S_1^c], [S_2]\right) = \frac{\partial Z^A}{\partial\left[S_2\right]} Z^I - Z^A \frac{\partial Z^I}{\partial\left[S_2\right]} \tag{SI-2}$$

alone determines the sign. We therefore must show that f changes sign as function of $[S_1^c]$. Specifically, the `XOR` gate requires

$$f > 0 \text{ for } [S_1^c] < [S_1^c]^*, \tag{SI-3}$$
$$f < 0 \text{ for } [S_1^c] > [S_1^c]^*, \tag{SI-4}$$

for some $[S_1^c]^*$ and all $[S_2]$.

The partition functions Z^A and Z^I for the Q_{UW} receptor are given by Eqs. 9-10 in the main text. Performing the derivatives in Eq. SI-2 reveals that f is a third order polynomial in $[S_1^c]$ in which all dependence on $[S_2]$ drops out. Only one root is potentially positive:

$$[S_1^c]^* = \frac{K_{1,W}^I K_{1,W}^A \left(K_2^I - K_2^A\right)}{K_{1,W}^I K_2^A - K_{1,W}^A K_2^I}. \tag{SI-5}$$

To satisfy Eqs. SI-3-SI-4, we require that the zeroth order term (the intercept) is positive and that the leading order term is negative; enforcing these conditions yields

$$K_2^I - K_2^A > 0, \tag{SI-6}$$
$$K_{1,W}^I K_2^A - K_{1,W}^A K_2^I > 0, \tag{SI-7}$$

which are in fact the precise conditions that maintain positivity of the root (Eq. SI-5). Parameters that satisfy these conditions enable the sign of $\partial p^A/\partial[S_2]$ to depend on constant $[S_1^c]$, which is one of the two conditions necessary to perform the `XOR` gate. Notably, Eq. SI-6 directly shows that the binding of ligand 2 to the W monomer in Q_{UW} in the active state is less likely than binding in the inactive state.

The second requirement is that the sign of $\partial p^A/\partial[S_1]$ depends on constant $[S_2^c]$. Specifically, as in Eqs. SI-3-SI-4, the `XOR` gate requires that the numerator $g([S_1], [S_2^c])$ of the derivative satisfies

$$g > 0 \text{ for } [S_2^c] < [S_2^c]^*, \tag{SI-8}$$
$$g < 0 \text{ for } [S_2^c] > [S_2^c]^*. \tag{SI-9}$$

for some $[S_2^c]^*$ and all $[S_1]$. Performing the derivative reveals that g is a second order polynomial whose coefficients depend on $[S_1]$. To satisfy Eqs. SI-8-SI-9, we again require that the intercept is positive and that the leading order term is negative; enforcing these conditions yields

$$h\left([S_1], K_{1,U}^I, K_{1,U}^A, K_{1,W}^I, K_{1,W}^A\right) > 0, \tag{SI-10}$$
$$K_{1,U}^I - K_{1,U}^A < 0, \tag{SI-11}$$

where the function h results straightforwardly from the derivative but is unwieldy, such that we do not reproduce it here. The roots of the polynomial $[S_2^c]^*$ are similarly unwieldy, but noting positivity requirements ($[S_2^c]^* > 0$, $K_{1,W}^I > 0$, $K_{1,U}^I > 0$, $K_2^I > 0$), parameter regimes can be derived that satisfy both Eqs. SI-6-SI-7 and Eqs. SI-10-SI-11 simultaneously. As an example we

present one possible regime here:

$$\frac{K^A_{1,W}}{K^I_{1,W}} < \frac{K^A_2}{K^I_2} < 1, \tag{SI-12}$$

$$0 < \frac{K^A_{1,W}}{K^I_{1,W}} \leq \frac{K^I_{1,U}}{K^I_{1,U} + K^I_{1,W}}, \tag{SI-13}$$

$$K^A_{1,W} + K^A_{1,U} > K^I_{1,U} + K^I_{1,W}. \tag{SI-14}$$

Eq. SI-12 states that the W monomer is activated by $[S_1]$ and $[S_2]$, and that activation by $[S_1]$ is stronger than activation by $[S_2]$. Note that for small concentrations of either $[S_1]$ or $[S_2]$, W is inactive. The two more interesting constraints are in Eq. SI-13 and Eq. SI-14. Eq. SI-14 states that $[S_1]$ bound to monomer U deactivates the receptor ($K^A_{1,U} > K^I_{1,U}$), since, from Eq. SI-12, we have seen that $K^A_{1,W} < K^I_{1,W}$. More importantly the deactivation of U by binding $[S_1]$ is stronger than the activation of W by $[S_1]$, and following Eq. SI-12, it is thus also stronger than activation of W by $[S_2]$. This is precisely the interference interaction as described in the main text. The last constraint, Eq. SI-13, provides the required binding strength of $[S_1]$ to U and W to satisfy all constraints. In the main text we argue that W should preferably bind $[S_2]$, such that in the presence of both ligand $[S_2]$ binds to W and $[S_1]$ binds to U with as result that Q_{UW} is inactive.

Here we have shown that the Q_{UW} receptor is capable of the nonmonotonic derivatives required by the `XOR` gate. This capability is necessary but not sufficient to perform the gate, as an ideal logic gate requires that the output be maximally high and low at the appropriate input values. Our numerical results, however, indeed confirm that the Q_{UW} receptor can perform the `XOR` gate.

SI. 3 Parameter sensitivity

In this section we discuss the sensitivity to parameter variation of the results at the six parameter sets φ_k shown in Fig. 3. Robustness against parameter fluctuations generally is considered an important quality of biochemical systems, due to stochastic nature of intra- and extracellular processes. If the observed logic gates only function within a very narrow parameter regime, this could lead to unreliable functioning.

Parameters are varied according to

$$\varphi^z_{\mathrm{new}} = \varphi^z_{\mathrm{old}} \left(1 + n^z\right) \tag{SI-15}$$

where n^z is the zth component of a uniformly distributed random vector $\mathbf{n}$ with norm $|\mathbf{n}| = \eta$. Under this implementation, η sets the average (root mean square) factor by which each parameter changes via $\langle \delta\varphi^z/\varphi^z \rangle = \eta/\sqrt{Z}$, where Z is the number of parameters. We sample 10^6 different vectors $\mathbf{n}$.

Sensitivity is measured by computing the fraction of new parameter sets for which, for each individual gate m, the relative change in fitness is less than a factor λ:

$$\frac{\left|F^{\mathrm{new}}_m - F^{\mathrm{old}}_m\right|}{F^{\mathrm{old}}_m} < \lambda \quad \forall\, m. \tag{SI-16}$$

Figure SI-5 reveals that for all φ_k, most random perturbations in which each parameter changes by an average of $\langle \delta\varphi^z/\varphi^z \rangle \sim 20\%$ change the fitness of none of the three logic gates by more than $\lambda = 10\%$.

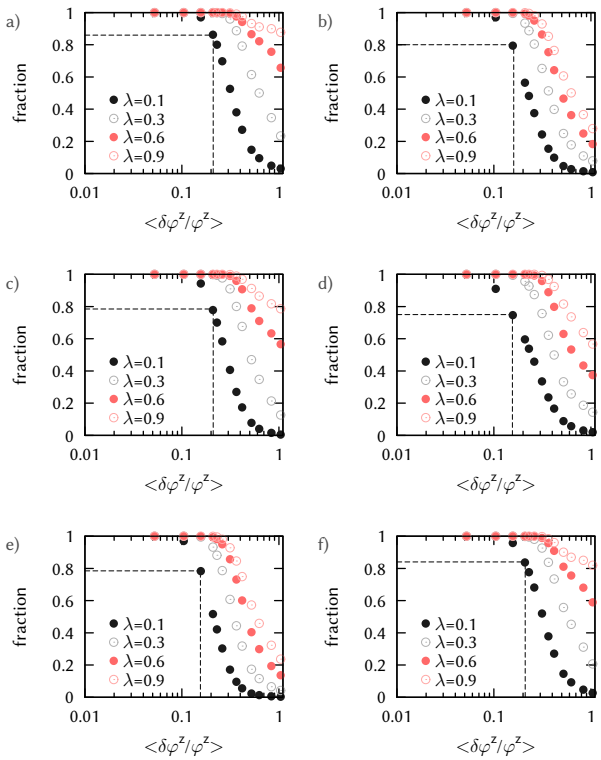

Figure SI-5: Robustness to parameter variation for the six parameter sets at which dimers can form three unique logic gates (Fig. 3): (a) φ_1, (b) φ_2, (c) φ_3, (d) φ_4, (e) φ_5, and (f) φ_6. An increase in $\langle \delta\varphi^z/\varphi^z \rangle$ reflects a larger range of parameter fluctuations and an increase in λ reflects a loosening of the robustness constraint. The dashed black lines indicate that a significant fraction of random perturbations in which each parameter changes by an average of $\langle \delta\varphi^z/\varphi^z \rangle \sim 20\%$ change the fitness of none of the three logic gates by more than $\lambda = 10\%$.

SI. 4 Further intuition behind functions accessible by recombination

In the main text, we provide the intuition behind how the first two groups of logic gates in Fig. 3 are performed by the corresponding receptors. Here, we provide similar intuition for the last four groups. Then, we argue why the six groups observed in Fig. 3 (and their counterparts obtained upon ligand exchange) are the *only* groups of three unique logic gates that one expects to observe under this model.

Parameter sets not discussed in the main text

Parameter set φ_3 (Fig. 3, third row) is similar to set φ_2 (see discussion of φ_2 in main text). In particular, receptor Q_{UW} performs the `XOR` gate in the same way. The difference between φ_3 and φ_2 is that ligand-bound V promotes activation instead of suppressing activation. Since ligand-bound W also promotes activation, this feature allows receptor Q_{WV} to perform the `OR` gate. However,

ligand-bound U suppresses activation more strongly than ligand-bound V promotes activation. This feature allows receptor Q_{UV} to perform the $\texttt{ANDN}_{S_1}$ gate, since only in the presence of ligand 2 and not 1 will activation be promoted via V and not suppressed via U.

Parameter set φ_4 (Fig. 3, fourth row) corresponds to a case where ligand-bound U and ligand-bound V both promote activation. This feature is sufficient for receptor Q_{UV} to perform the `OR` gate. Furthermore, ligand 2 binds more strongly to V than to W, and ligand-bound W suppresses activation more strongly than ligand-bound V promotes activation. These features allow receptor Q_{WV} to perform the $\texttt{ANDN}_{S_1}$ gate, since only in the presence of ligand 2 and not 1 will activation be promoted via V and not suppressed via W. Finally, (i) ligand 1 binds more strongly to W than to U, (ii) ligand 2 binds more strongly to W than ligand 1 does, and (iii) ligand-bound U promotes activation more strongly than ligand-bound W suppresses activation. These three features allow receptor Q_{UW} to perform the `AND` gate: when ligand 1 is present alone, feature (i) results in suppression via W; when ligand 2 is present alone, it only binds to W, resulting in suppression; and when both ligands are present, features (ii) and (iii) cause ligand 2 to bind to W, forcing ligand 1 to bind to U and thus activating the receptor.

Parameter set φ_5 (Fig. 3, fifth row) is once again similar to set φ_2. In particular, receptors Q_{WW} and Q_{UW} perform the `OR` gate and the `XOR` gate in the same way, respectively. Additionally, ligand 1 suppresses activation via U more strongly than ligand 2 promotes activation via V. This feature allows receptor Q_{UV} to perform the $\texttt{ANDN}_{S_1}$ gate, since only in the presence of ligand 2 and not 1 will the receptor be active.

Parameter set φ_6 (Fig. 3, sixth row) is similar to set φ_1 (see discussion of φ_1 in main text). In particular, receptors Q_{WW} and Q_{UW} perform the `AND` gate and the `OR` gate in the same way, respectively. Additionally, ligand 2 suppresses activation via V more strongly than ligand 1 promotes activation via U. This feature allows receptor Q_{UV} to perform the $\texttt{ANDN}_{S_2}$ gate, since only in the presence of ligand 1 and not 2 will the receptor be active.

Figure 3 is exhaustive

Here, we argue why the groups observed in Fig. 3 (and their counterparts obtained upon ligand exchange) are the only groups of three unique logic gates that one expects to observe under this model. The overall logic is presented first, with the arguments subsequently given in subsections.

There are 4 ways to choose a group of three from the four functional dimers Q_{WW}, Q_{UW}, Q_{WV}, and Q_{UV} to perform the three unique logic gates: $\{Q_{\mathrm{WW}}, Q_{\mathrm{UW}}, Q_{\mathrm{WV}}\}$, $\{Q_{\mathrm{UW}}, Q_{\mathrm{WV}}, Q_{\mathrm{UV}}\}$, $\{Q_{\mathrm{WW}}, Q_{\mathrm{UW}}, Q_{\mathrm{UV}}\}$, and $\{Q_{\mathrm{WW}}, Q_{\mathrm{WV}}, Q_{\mathrm{UV}}\}$. The last two groups are symmetric upon ligand exchange; we therefore consider only the first three groups.

The first group is $\{Q_{\mathrm{WW}}, Q_{\mathrm{UW}}, Q_{\mathrm{WV}}\}$. As shown in the main text, receptor Q_{WW} is capable of performing an `AND` gate, an `OR` gate, or an `ANDN` gate, but not an `XOR` gate (Fig. 2). If receptor Q_{WW} performs an `AND` gate, receptor Q_{UW} can perform an `ANDN` gate or an `OR` gate, but not an `XOR` gate (Argument 1). Receptor Q_{WV} then performs the `OR` gate or the `ANDN` gate, respectively (it also cannot perform an `XOR` gate by the same argument). These two possibilities are represented by parameter set φ_1 (Fig. 3) and its counterpart upon ligand exchange. If receptor Q_{WW} performs an `OR` gate, receptor Q_{UW} can perform an `ANDN` gate or an `XOR` gate, but not an `AND` gate (Argument 2). Receptor Q_{WV} then performs the `XOR` gate or the `ANDN` gate, respectively (it also cannot perform an `AND` gate by the same argument). These two possibilities are represented by parameter set φ_2 (Fig. 3) and its counterpart upon ligand exchange. If receptor Q_{WW} performs an `ANDN` gate, three

unique gates cannot be performed (Argument 3). Therefore, this group is exhaustively represented by φ_1 and φ_2.

The second group is $\{Q_{\mathrm{UW}}, Q_{\mathrm{WV}}, Q_{\mathrm{UV}}\}$. As shown in the main text, receptor Q_{UV} is capable of performing an `ANDN` gate, an `OR` gate, or an `AND` gate, but not an `XOR` gate (Fig. 2). If receptor Q_{UV} performs an `ANDN` gate, receptor Q_{UW} can perform an `XOR` gate or an `OR` gate, but not an `AND` gate (Argument 4). Receptor Q_{WV} then performs the `OR` gate or the `XOR` gate, respectively (it also cannot perform an `AND` gate by the same argument). These two possibilities are represented by parameter set φ_3 (Fig. 3) and its counterpart upon ligand exchange. If receptor Q_{UV} performs an `OR` gate, receptor Q_{UW} can perform an `AND` gate or an `ANDN` gate, but not an `XOR` gate (Argument 5). Receptor Q_{WV} then performs the `ANDN` gate or the `AND` gate, respectively (it also cannot perform an `XOR` gate by the same argument). These two possibilities are represented by parameter set φ_4 (Fig. 3) and its counterpart upon ligand exchange. If receptor Q_{UV} performs an `AND` gate, three unique gates cannot be performed (Argument 6). Therefore, this group is exhaustively represented by φ_3 and φ_4.

The third group is $\{Q_{\mathrm{WW}}, Q_{\mathrm{UW}}, Q_{\mathrm{UV}}\}$. We note that this group is different from the first two groups, since it does not contain the two receptors Q_{UW} and Q_{UV} which are symmetric upon ligand exchange. As shown in the main text, receptor Q_{WW} is capable of performing an `AND` gate, an `OR` gate, or an `ANDN` gate, but not an `XOR` gate (Fig. 2). If receptor Q_{WW} performs an `AND` gate, receptor Q_{UW} can perform an `ANDN` gate or an `OR` gate, but not an `XOR` gate (Argument 1). If receptor Q_{UW} performs an `ANDN` gate, receptor Q_{UV} cannot perform an `OR` gate (Argument 7); since receptor Q_{UV} also cannot perform an `XOR` gate (Fig. 2), three unique gates cannot be performed. Therefore, receptor Q_{UW} must perform an `OR` gate, leaving receptor Q_{UV} to perform an `ANDN` gate. This possibility is represented by parameter set φ_6 (Fig. 3). If receptor Q_{WW} performs an `OR` gate, receptor Q_{UW} can perform an `ANDN` gate or an `XOR` gate, but not an `AND` gate (Argument 2). If receptor Q_{UW} performs as an `ANDN` gate, receptor Q_{UV} cannot perform an `AND` gate (Argument 8); since receptor Q_{UV} also cannot perform an `XOR` gate, three unique gates cannot be performed. Therefore, receptor Q_{UW} must perform an `XOR` gate, leaving receptor Q_{UV} to perform an `ANDN` gate. This possibility is represented by parameter set φ_6 (Fig. 3). If receptor Q_{WW} performs as an `ANDN` gate, three unique gates can not be performed (Argument 9). Therefore, this group is exhaustively represented by φ_5 and φ_6.

This completes the logic arguing that the groups observed in Fig. 3 are exhaustive.

Argument 1

If receptor Q_{WW} performs an `AND` gate, ligand 2 alone does not promote activation when binding to monomer W. Therefore, because ligand 2 does not bind to monomer U, the receptor Q_{UW} is always inactive if ligand 2 is present alone. This behavior is inconsistent with the logic of the `XOR` gate.

Argument 2

If receptor Q_{WW} performs an `OR` gate, ligand 2 alone promotes activation when binding to monomer W. Therefore, because ligand 2 does not bind to monomer U, the receptor Q_{UW} is always active if ligand 2 is present alone. This behavior is inconsistent with the logic of the `AND` gate.

Argument 3

If receptor Q_{WW} performs an `ANDN` gate, receptors Q_{UW} and Q_{WV} can each perform neither an `XOR` gate nor an `AND` gate, thereby preventing the group $\{Q_{\mathrm{WW}}, Q_{\mathrm{UW}}, Q_{\mathrm{WV}}\}$ from performing three unique gates. The reason is straightforward: if receptor Q_{WW} performs an `ANDN` gate, one ligand must suppress activation via W while the other ligand promotes activation via W. This feature immediately excludes the `XOR` gate since, as described in the main text, an `XOR` gate requires both ligands to promote activation via W. This feature also excludes an `AND` gate since, as also described in the main text, an `AND` gate requires either that activation via W is promoted only weakly or that both ligands suppress activation via W. In the first case, activation of receptor Q_{UW} (or Q_{WV}) is only achieved cooperatively when both ligands are present. In the second case, activation is achieved with both ligands present via U (or V) due to an interference effect similar to that underlying the `XOR` gate (see discussion of parameter set φ_4 above).

Argument 4

If receptor Q_{UV} performs an $\texttt{ANDN}_{S_1}$ gate, Q_{UW} cannot function as an `AND` gate. To function as an `AND` gate (see Eq. 9), this requires that $\omega_0 K_{1,U}^A K_2^A \ll K_{1,U}^I K_2^I$, while $\omega_0 K_{1,U}^A \gg K_{1,U}^I$ and $\omega_0 K_2^A \gg K_2^I$. This conditions cannot be satisfied simultaneously.

Argument 5

If receptor Q_{UV} performs an `OR` gate, ligand 1 activates the receptor via U. However, for receptor Q_{UW} to perform the `XOR` gate, ligand 1 must suppress activation via U, as described in the main text.

Argument 6

If Q_{UV} functions as an `AND` gate U is activated by S_1 and V is activated by S_2, but both activation biases alone are insufficient to activate the receptor. This excludes the formation of a `XOR` gate for either the Q_{UW} or Q_{WV}. As we have discussed in the previous section, the `XOR` gate is obtained by the deactivation of U (V) by ligand S_1 (S_2). However, it is possible that Q_{UW} is a `OR` gate, while Q_{WV} is a `ANDN` gate. The Q_{UW}-`OR` gate requires that W is activated by S_2 and S_1, since monomer U is not active in the presence of S_1. The Q_{WV}-`ANDN` gate requires that W is strongly deactivated by S_1. These two conditions on W are mutually exclusive.

Argument 7

If receptors Q_{WW} and Q_{UW} perform an `AND` gate and an `ANDN` gate, respectively, the `ANDN` gate must be $\texttt{ANDN}_{S_1}$, not $\texttt{ANDN}_{S_2}$. The reason is that the `AND` gate requires ligand 2 to promote activation via W, while the $\texttt{ANDN}_{S_2}$ gate requires ligand 2 to suppress activation via W. Then, if Q_{UW} indeed performs the $\texttt{ANDN}_{S_1}$ gate, receptor Q_{UV} cannot perform an `OR` gate. The reason is that the `AND` and $\texttt{ANDN}_{S_1}$ gates require ligand 1 to suppress activation via U and not via W, while the `OR` gate requires ligand 1 to promote activation via U.

Argument 8

If receptors Q_{WW} and Q_{UW} perform an `OR` gate and an `ANDN` gate, respectively, the `ANDN` gate must be $\texttt{ANDN}_{S_1}$, not $\texttt{ANDN}_{S_2}$. The reason is that the `OR` gate requires ligand 2 to promote activation via W, while the $\texttt{ANDN}_{S_2}$ gate requires ligand 2 to suppress activation via W. Then, if Q_{UW} indeed performs the $\texttt{ANDN}_{S_1}$ gate, receptor Q_{UV} cannot perform an `AND` gate. The reason is that the `OR` and $\texttt{ANDN}_{S_1}$ gates require ligand 1 to suppress activation via U and not via W, while the `AND` gate requires ligand 1 to promote activation via U.

Argument 9

If receptor Q_{WW} performs an `ANDN` gate, receptor Q_{UW} cannot perform a `XOR` gate gate, since this requires that both ligands activate W. If receptor Q_{WW} performs an $\texttt{ANDN}_{S_1}$ gate, receptor Q_{UW} cannot perform an `AND` gate, since Q_{UW} is active if only ligand 2 is present. If receptor Q_{WW} performs an $\texttt{ANDN}_{S_1}$ gate, receptor Q_{UW} can perform an `OR` gate if ligand 1 activates U more strongly than it deactivates W. However, receptor Q_{UV} is then always active if ligand 1 is present, and this is inconsistent with the logic of the `AND` gate. If receptor Q_{WW} performs an $\texttt{ANDN}_{S_2}$ gate, receptor Q_{UW} cannot perform an `AND` gate, since Q_{UW} is active if only ligand 1 is present (ligand 1 activates U) or Q_{UW} is never active (ligand 1 deactivates U more strongly than it activates W). If receptor Q_{WW} performs an $\texttt{ANDN}_{S_2}$ gate, receptor Q_{UW} can perform an `OR` gate, if (i) ligand 1 activates U and (ii) in the presence of small ligand 1 and an abundance of ligand 2 the receptor Q_{UW} is active. However, receptor Q_{UV} is then always active if ligand 1 is present and this is inconsistent with the logic of the `AND` gate.